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DEVELOPMENTS IN DELAYED-TYPE HYPERSENSITIVITIES: 1950–1975

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Significant developments during the last 25 years are discussed and interpreted. The following areas of delayed hypersensitivity are included: the mode of active sensitization to simple allergenic chemicals; evidence for anamnestic responses; cell types and cell–cell interactions via lymphokines; function of skin and lymphatics, and the role of the carrier in initial sensitization to allergenic chemicals; acquired tolerance; transfer factor. Some prognostications for the future are attempted.

BACKGROUND

Without benefit of government support, the years before 1950 witnessed an emergence of significant knowledge which would influence the subsequent quarter-century. By 1950, quantitative immunochemistry had been established even though the various classes of immunoglobulins were still unknown. Pneumococcal "types" had been transformed, that is, the synthesis of new capsular polysaccharides had been induced by the addition of pneumococcal extracts, and with this achievement came an early but limited recognition of the complexity and role of DNA in biology. The complement system was lusty but not yet out of diapers.

Between 1935 and 1950, significant facts about contact hypersensitivity had been disclosed by means of simple chemicals. The results of Landsteiner and Jacobs [1] in their study of the sensitizing capacity of nitro-substituted and chloro-substituted benzenes in guinea pigs had shown that this class of chemicals sensitizes only when the particular compound possesses a labile substituent which allows hapten–protein complexes to form, and that carrier protein is represented by the host's own structural elements. These results were later embellished by Eisen, Orris, and Belman [2], who found that the ability not only to sensitize but also to elicit reactions requires compounds with labile substituents. Delayed-type hypersensitivities (D-H) had been transferred to normal, outbred, recipient guinea pigs by means of white cells from sensitized animals, both for allergic contact dermatitis and for cutaneous hypersensitivity to tuberculin [3,4]. One could then appreciate how transfers of unclarified blister fluids, secured just below the sites of positive contact reactions on human beings, had been irregularly reported to transfer contact dermatitis in the Urbach–Königstein technique [5].

Another subject had been introduced, seemingly of no interest to dermatologists or allergists since the experiments were being done with guinea pigs. When normal guinea pigs were fed sensitizing chemicals in triglyceride oil before sensitizing courses with the same chemical had been undertaken, a state of specific unresponsiveness to the acquisition of D-H could be established (see *The Unresponsive State* below).

Again, Jules Freund [6] had confirmed the findings of Coulaud [7] and Saenz [8,9], namely, that pronounced sensitivity to tuberculin arises when heat-killed virulent mycobacteria are suspended in hydrocarbon or paraffin oil before injection. Shortly afterwards, Freund made his unique contribution, an expansion of earlier experiments by Dienes and Schoenheit [10], i.e., the incorporation of water-soluble or water-suspended material dispersed *within* the paraffin oil–mycobacterial continuous phase by means of special water-in-oil (W/O) emulsifiers (Aquaphor, Protegin-X, Falba, Arlacel A). Later, Freund with McDermott [11] showed that such dispersed antigens give rise to D-H and to an extraordinary synthesis of precipitating antibody by guinea pigs, and Landsteiner and Chase (review in [12]) induced contact sensitivity to picryl chloride in guinea pigs by se-

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Abbreviations:

B-: bone marrow-derived
CBH: cutaneous basophil hypersensitivity
CFA: complete Freund's adjuvant
CMI: cell-mediated immunity
D-H: delayed-type hypersensitivity
DNCB: dinitrochlorobenzene
DNFB: dinitrofluorobenzene
DNP: dinitrophenylated
DMSO: dimethylsulfoxide
EAC: erythrocyte–antibody–complement complex
IFA: incomplete Freund's adjuvant
MIF: macrophage inhibition factor
MLC: mixed lymphocyte culture
NDMA: *p*-nitrosodimethylaniline
PCl: picryl chloride
PMN: polymorphonuclear leukocyte
T-: thymus-derived
TF: transfer factor.

quential intraperitoneal injections of dead tubercle bacilli in paraffin oil and of aqueous suspensions of the picrylated ghosts of guinea-pig erythrocytes.

If taken in strict chronologic order, the sweep of events over 25 years in any given discipline would be a jumble because of the various facets of delayed hypersensitivity. Taken together, they require a separate volume such as Turk's [13]. We think at once of primary homograft rejection, poison ivy, and other contactants, hypersensitivity in mycobacterial and fungal diseases, rare reactions to medicaments, and the story of transfer factor. Obviously compartmentalization is needed even for this superficial review. Furthermore, studies on experimental animals, chiefly the guinea pig, will be cited whether or not parallel findings exist or have been sought for in man.

ACTIVE SENSITIZATION

Methods of Sensitizing

Various procedures have been developed to induce allergic contact-type sensitivity to chemical agents (Tab. I). Classically, for both man and guinea pigs, the skin route (single or repeated application on or into the skin) has played a dominant role. The value of pre-irritation of the skin is firmly established for both man and animal. Pre-irritation techniques have been used vigorously to better exploit the use of the guinea pig for prospective studies of materials intended for human use [14–16].

TABLE I. *Sensitization of guinea pigs to simple chemicals*

1. Percutaneous absorption
   - Salves (Vaseline, lanolin, Orabase) and oils
   - Closed patches (Magnusson)
   - Local irritation as an assist:
     - Self-irritant concentrations, e.g., 2% DNCB in ethanol
     - Incorporation of irritant, as organic peroxides
     - Applications of dilute cantharidin (Landsteiner)
     - Freon 12 on human subjects (Kligman)
     - Dry ice burns (Maguire)
     - Skin masceration by 5% SDA (Magnusson and Kligman)
   - Dimethylsulfoxide (DMSO) solutions
2. Intradermal injections of microgram amounts
   - Typical solvents: 4% alcohol in saline, corn oil, paraffin oil
3. Use of mycobacteria-liquid hydrocarbon suspensions
   - W/O emulsion with emulsifier ("CFA")
     - Simple chemical in aqueous phase
     - Simple chemical in hydrocarbon phase
     - Hapten–carrier complexes in aqueous phase
   - Mycobacteria in paraffin oil i.d., followed at 24 hr by i.d. allergen into sites (Split-adjuvant technique of Maguire)
   - Mycobacteria in paraffin oil i.d., followed by dry ice application, salve, and occlusive dressing (Maguire)
4. "Combination"—Hapten–carrier complex in CFA, then contact tests with hapten on days 10 and 17
5. Intradermal injections of hapten–epidermal tissue complexes (Chase and Kawata)

The other principal technique makes use of "complete" Freund's adjuvant (CFA) in which W/O emulsions are injected, the mycobacterial cells being in the continuous phase of paraffin oil. This assist works on a different principle since attacking cells carry minute droplets of emulsion throughout the body with slow release of the contained aqueous droplets and well-nigh permanent deposition of mycobacteria and oil in the tissues [17]. As stated above, hypersensitivity to tuberculin is established simultaneously. Besides the depot role of CFA, there is evidence for a direct and early stimulation of lymphoid cells by mycobacteria [18].

Sensitization to microbial agents is usually effected, as in man, by invasion of tissues with living organisms or by supplying these killed, in antigenic dosage. In particular instances, a temporary state of delayed hypersensitivity has been facilitated by using "incomplete" Freund's adjuvant (IFA, that is, paraffin oil-plus-Arlacel A, but without mycobacteria) as a depot for antigen The term "Jones-Mote" is often borrowed by immunologists to describe this sensitivity.

Skin grafts placed upon outbred or histoincompatible animals represent another mode of developing D-H and of securing lymphocytes which upon transfer will induce speedy rejection of an established homograft. The parallelism with allergic contact dermatitis was clearly established in experiments on guinea pigs, in which tests for D-H can be made readily. Thus, intradermal injection had shown that animals which had rejected homografts had become sensitized to living cells or extracts of tissues of the donor animal; visually and histologically the D-H responses were typical. This test is termed the direct hypersensitivity reaction [19,20]. The reverse procedure, i.e., injecting the white cells of the rejecting animal into the skin of the original homograft donor, is naturally positive also; basically, it is a graft-versus-host reaction [21].

Anamnestic Responses in Delayed Hypersensitivity

"Boosting" the titer of immunoglobulins by reinjecting antigen is the classical type of anamnestic response, yet boosting also occurs, although seldom recognized, in D-H. In the simplest case, guinea pigs receiving an intradermal injection of dinitrochlorobenzene (DNCB) but not scoring highly in the subsequent contact test are often found to display much more intense reactions in a second contact test. Investigation showed that the first test is a prerequisite and that it should be made within 3 weeks of the sensitizing injection. After the second test is appropriately made within

2 to 3 weeks, contact reactivity of long duration sets in and often remains at high level for over a year.

Pronounced examples of ascending sensitivity induced by subsequent contact testing are encountered when hapten-homologous erythrocyte stromata are injected in CFA [12,22]. The animal may finally react to contact with as little as 0.0025% solution in triglyceride oil. In the split-adjuvant technique introduced by Maguire and Chase [23], picric acid is injected intradermally and, later, 2.5 μg of mycobacteria in 0.05 ml of paraffin oil are injected into the same sites. The stage is set for a marked increase in contact sensitivity when 2 or 3 spaced contact tests are made with picric acid [24,25]. Indeed, peritoneal exudate cells obtained early and late in the sensitizing routine differed in transfer capacity: the early cells transferred the indolent, slow responses of their donors; and the late cells, a sharply ascending, intense reactivity. Transferring fewer of the late cells caused decreasing reactions, but of a constant type. Since dilution did not imitate the response to early cells, there had been a qualitative, not a quantitative, alteration in the lymphocytes during the anamnestic responses [25].

Theories of the mechanism must take into account a possible switch in hapten–carrier as one element. Initial intradermal injections might find a dermal sensitizing carrier and contact-testing might utilize an epidermal carrier (see *The Unresponsive State*). But the stepwise boosts by the several contact tests remain true anamnestic responses.

Histology of D-H Reactions

Sequential biopsies in animals after skin tests show lymphocytes and other cells emerging from the lumen of blood vessels and migrating upwards towards and beyond the dermal–epidermal junction to the site of the test application; many clusters remain around vessels in the dermis. Spongiosis and edema are observed in the developed reaction. Typical examples are given by Macher and by Fisher for DNCB contact tests [26,27]. Lymphocytes, monocytes, histiocytes, polymorphonuclear cells (PMNs) and a scattering of eosinophils are seen; special staining is required to reveal the basophils. In the special case of intense contact-sensitivity to picric acid, subepidermal abscesses of nearly pure PMNs are found [24]. In recent studies by Dvorak and his group [28,29], the elegant techniques of fixation, embedding and of staining have led to a recognition of basophils in D-H reactions and have confirmed the earlier study of Per Wolf-Jürgensen [30] with the skin-window technique. At first, basophils were thought to be characteristic of Jones-Mote reactions; further studies have shown that they occur in various numbers in all D-H reactions, but relatively fewer in tuberculin test sites. Fibrin, usually a prominent component of D-H, occurs principally intervascularly in the reticular dermis, but does not approach the vessels or cuffs of the perivascular cells [31].

An elaborate and highly informative study of contact reactions in man has been made by Dvorak et al [32], who based their result on a microscopic study of 180 biopsies, many serial, from volunteers sensitized to DNCB. Basophils were observed in 91% of the positive reactions at their peak, fewer than those in sensitized guinea pigs; mast cells were also evident. Skin testing with microbial antigens had induced basophils in 61% of the biopsies, less than in the guinea pig. The role of basophils is not known; instead of explosive degranulation, bits of granules appear to be released slowly and many granules remain intact. Now that guinea-pig basophils can be purified, closer studies may be possible [33].

Jones-Mote Reactions

An evanescent period, during which D-H reactions can be demonstrated, occurs 5 or 6 days after, for example, injection of guinea pigs with ovalbumin in IFA. These delayed reactions are usually of low intensity [28,34]; suppressor B-cells are thought to play a role since the reactions are much

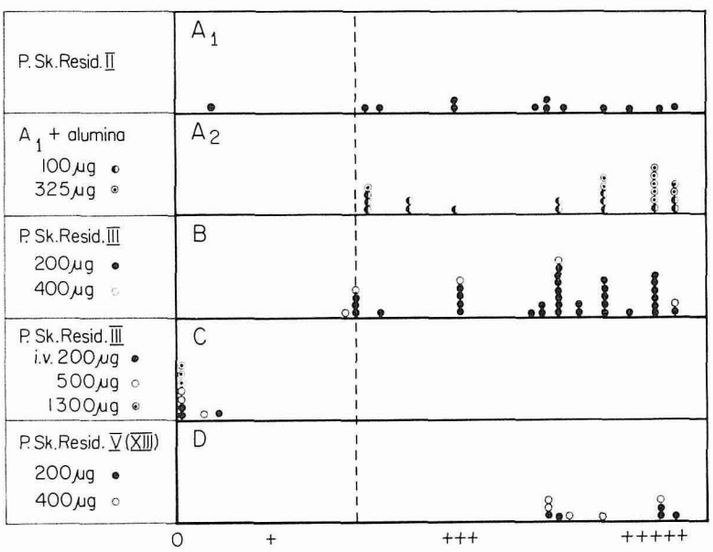

FIG. 1. Contact sensitization induced by picrylated epithelial residues. The baseline indicates the intensity of reaction at 24 hr after contact tests with 1% picryl chloride in corn oil were applied on days 5 or 6 following the intradermal injections described in the text. Gradings: +, confluent, faintly pink reaction, only slightly thickened as palpated; ++, pale pink, moderately thickened; +++, between pale pink and pink, indurated; ++++, solid, strong pink, well indurated; +++++, bright pink, markedly indurated. The *vertical dashed line* is set arbitrarily at ++ in order to exclude counting the few animals which may show high irritative or "toxicity" reactions. With each group tested, naive animals of same sex and weight are tested in parallel. Experimental animals with contact reactions rated as +++ to +++++ can be accepted without question as being highly sensitive. *Groups* A_1 and A_2: a single picrylated skin extract injected alone or with alumina; *Group B*: another preparation, injected intradermally alone; *Group C*: the same preparation injected intravenously as control; *Group D*, a third preparation, prepared from epithelial residues of Wright's Family XIII guinea pigs.

enhanced if cyclophosphamide (which suppresses B-cells) is given 3 days before sensitization [35,36]. Dvorak et al [28,29], using thin plastic-embedded sections, discovered large numbers of basophils in such test sites and proposed a special name, cutaneous basophil hypersensitivity (CBH). This term must be taken only in a relative sense since basophils appear in all sorts of D-H reactions. Currently, Dvorak retains CBH even for allergic contact hypersensitivity and believes that only reactivity induced by CFA is actually classic D-H. This extraordinary conclusion would put D-H apart from the bulk of delayed-type reactions, which would then be known as CBH. In Jones-Mote reactions, the feeblest of the D-H reactions, cellular transfer is demonstrable. Furthermore, in all CBH reactions, the lymphocyte remains the controlling cell. Since cyclophosphamide acts on B-cells and Jones-Mote sensitizations are stronger when it has been used (see above), it seems reasonable to grade D-H reactions on a scheme of intensities and to welcome basophils as cells whose role is under current investigation.

Basophils also occur in relation to antibody-induced reactions [38]. Askenase [39] sensitized guinea pigs with a substituted oxazolone in a manner that led to the appearance of circulating antibody, probably IgG_1. When the serum of these animals was transferred to a recipient it gave rise to the same slow binding of antibody at test sites that I have observed and that can imitate a weak delayed reaction.

ROLE OF THE SKIN IN ALLERGIC CONTACT DERMATITIS

Sensitization by Simple Allergenic Chemicals

In simple sensitization by allergenic chemicals, that is, without the use of adjuvant, a deposition of the sensitizer in sites other than the dermis usually fails to sensitize. Yet sensitization is possible via other epithelia—vagina, uterus, colon—and the sensitized animal will exhibit typical contact reactions on the skin [26]. Since hapten sensitizes after it couples with carrier, which is so altered by the attachment of a sufficient number of haptenic groups per mole as to be recognized as "non-self," many attempts have been made to produce a sensitizing complex in vitro. Dinitrophenylated serum albumin or entire human serum proteins, used in conjunction with CFA, induced contact sensitivity only marginally; moreover, such products were unreactive in skin tests of persons sensitive to DNCB or dinitrofluorobenzene (DNFB).

In place of serum proteins, soluble proteins extracted directly from guinea-pig skin and coupled with picryl chloride (PCl) and injected in IFA gave a glimmer of sensitization [40,41]. (Some proteins are found only in the dermis and certain others are shared by other organs.) Finally, Chase and Kawata [42] showed that homologous epithelial scrapings are the proper carrier for hapten coupling. These were defatted, thoroughly extracted, and subdivided by sonication so that they could be injected into the skin. Such material was coupled with PCl or DNFB, and excessive unreacted hapten was removed before it was used as a direct sensitizer (Fig. 1). Five simultaneous intradermal injections of 0.05 ml containing picrylated skin particles (10–400 μg total dose) were made in a circular pattern on one shoulder. When a contact test was applied 5 days later and read the following day, contact sensitivity was highly developed. The elapsed time between injection and contact testing was less than when PCl is injected intradermally since the free hapten requires time to couple. Moreover, the reactions were more brilliant, a point to be mentioned later. Dinitrophenylated skin particles bring a reasonable degree of contactant sensitivity to DNFB or DNCB to half the injected animals, sufficient to confirm the principle. This experiment shows that intradermally deposited, insoluble skin particles provide a complex that sensitizes without requiring the animal to encounter free hapten. The bulk of the injected material must stay in the skin since marked flaring occurs at the intradermal injection sites around day 4 or 5, but undoubtedly some of it is transported elsewhere.

The Role of Lymphatics

When the sensitizer is placed upon an isolated island of skin [43] or a stalk of isolated tissue [44], sensitization occurs only if the lymphatics are patent. Conversely, when foreign tissue is grafted to the alymphatic cheek pouch of the hamster, which is called a "privileged site," delayed sensitivity does not occur [21].

The lymphatic channel draining the cutaneous site of deposited allergen was long thought to serve as a passage for allergen—or antigen from a skin homograft—to the draining nodes, where the actual sensitizing sequence took place [45,46]. But this is evidently not so for sensitization with allergenic chemicals of the DNCB or substituted oxazolone type except where CFA has been used. Sensization occurs in the skin at the site where hapten becomes bound to the structural tissue; lymph cells arrive at that "training field" by traversing the walls of blood vessels and, once there, somehow acquire the ability to recognize the hapten-tissue complex. The patent lymphatic serves rather as a path whereby lymphocytes escape to the nodes, where the cells divide and clonalize and are ready to respond when the same hapten-tissue complex is formed anew by a subsequent contact test. Evidence for this theory comes from the work of Egon Macher in my laboratory, who used ^{14}C-labeled PCl and DNCB precisely injected in 0.01 ml amount into the ear of guinea pigs, and avoided using large dosages that would provide extrasensitizing amounts. Ear excision showed that free hapten rapidly left the injected site through the walls of blood vessels, not through the lymphatics. When

99% had drained away and therefore had already become "available" to the animal, excision of the ear with its small residuum of bound hapten still blocked sensitization [17,47,48]. Sensitization via radiolabeled urushiol may be an exception [49], but we think not. Evidently urushiol, the active principle of poison ivy, becomes altered very slowly in its catechol groups and is found widespread through the body. The fact, however, does not preclude the theory of "peripheral sensitization," which is the term proposed by Medawar [50] to suggest that homograft rejection is the result of attack by host lymphocytes which have acquired their recognition of "non-self" within the very graft. A probable exception to the theory of peripheral sensitization is the induction of sensitivity with CFA, where (it may be surmised) local sites around deposited mycobacteria, the chemistry of which induces cellular proliferation, serve as training fields [17].

In skin homografting, where patency of the lymphatic pathway from the graft is necessary for its rejection, and obliteration of the pathway prolongs survival, it was again concluded that the patent lymphatic allows the "trained" lymphocytes to escape [51].

As a final step, Macher and Sommer attempted to "train" lymphocytes to recognize antigen in vitro [52]. Eventually, Polak and Macher [53] reported that some measure of success seemed attainable; further discussion will be found under *The Unresponsive State.*

Sensitization with Dinitrophenylated (DNP) Amino Acids and Simple DNP Complexes

Reports that contact sensitivity will arise by simple intradermal injections of DNP-amino acids in the absence of adjuvants will be mentioned briefly. An excellent study with 35 such compounds [54] reports that 10 were regularly immunogenic, and 15 were variably immunogenic, i.e., were negative when purchased from source No. 1 and immunogenic from source No. 2. Such source-dependent results are interpreted as dependent upon contamination with some immunogenic impurity possessing DNP specificity and being carried along through the commercial processing steps. Of the 10 immunogenic compounds, di-DNP-L-histidine received most study: the authors concluded that a "transconjugation" phenomenon occurs with the positive compounds, a switch of the DNP group directly from the amino acid carrier to adjacent proteins, and evidence is offered in support. The in vivo fate of the most-quoted D-H immunogen, *p*-azobenzene arsonate-tyrosine, is apparently not elucidated.

ROLE OF THE HAPTEN CARRIER IN DETERMINING THE SPECIFICITY OF DELAYED-TYPE HYPERSENSITIVITY

When haptens are attached in vitro to a series of unrelated proteins and sensitization is achieved with one of these compounds via CFA, subsequent testing, which requires intradermal injections, shows that reactivity is confined to the particular complex used to sensitize; the other carriers, bearing the same hapten, cause no reactions [55]. This finding led to the dictum that specificity in D-H is determined by the entire complex, but that IgG_1 and IgG_2 antibodies to the same complex cross-react with all the complexes by virtue of the common hapten group. The principle was confirmed by David et al who used carriers of widely different provenance to produce MIF in vitro [56].

I gave an example above in which different carrier structures within an animal may be engaged depending upon the route chosen for sensitizing. Another example is worth citing. Intradermal (i.d.) injection of picric acid-insolubilized protein leads to a D-H reaction that is confined to i.d. testing with picric acid; only the i.d. sensitivity is transferrable with cells, and recipients do not react to contact testing with picric acid [25]. But in sensitization to picric acid by the split-adjuvant technique [24], in which both i.d. and contact routes are utilized, cellular transfer effects a sensitivity which is expressed in both i.d. and contact tests. The finding again suggests that the carriers utilized in i.d. injection and in application to the epidermis are sufficiently different to modify the specificity observed.

CELLS AND DELAYED HYPERSENSITIVITY

The first clear indication of the role of lymphocytes in delayed hypersensitivity came in 1942 for allergic contact dermatitis of the guinea pig [3] and in 1945 for tuberculin hypersensitivity [4] when cellular transfer was demonstrated in outbred guinea pigs. Systemic reactions to tuberculin after transfer were first reported by Kirchheimer and Weiser in 1947 [57]. The highly erythematous color of contact reactions is a vascular "overlay," which disappears when the animal is exsanguinated to reveal the palpable cellularity within the dermis and epidermis. The mouse and rat also develop delayed reactions, but they do not exhibit the vascular component.

Cellular Transfer

Cellular transfer of delayed hypersensitivity has become routine in animal experiments. Purists (I am not among them) reserve the term "adoptive immunity" for cellular transfers made between isologous or near-isologous stocks in which rejection phenomena are hardly evident. But for many purposes, the rejection of cells transferred between outbred guinea pigs is desirable. With contact sensitivity, reactions occur at or before 19 hr if the contact test was given 3 hr before the cells were injected. Wesslén showed that transfer between rabbits also occurred with thoracic duct cells and thus narrowed the cell-type involved [58].

By cellular transfer between monkeys, Hensley, Fink, and Barboriak [59] have investigated the delayed hypersensitivity aspects of allergic pneumonitis. The cell sources can be peripheral blood,

peritoneal exudate cells, and lymphoid cells from nodes or spleens. Peritoneal exudate cells appear to be almost totally devoid of B-cells since immunoglobulin is not synthesized by them [22].

Emphasis on the role of cells in "transplantation immunity" led to the early introduction of the unfortunate term CMI (cell-mediated immunity), which should be replaced by CMH (cell-mediated hypersensitivity, the actual underlying state). I reserve the term CMI only for cases of immunity induced by transferred cells to infectious agents, such as virulent *Listeria monocytogenes* [60,61]. This relation of CMI to resistance to infection has been studied under the WHO [62].

Correlates of Delayed Hypersensitivity in Vitro

It was natural to try to determine whether cells of sensitive animals respond in vitro to antigen, but this hypothesis could not be tested directly with simple chemical allergens. Delayed hypersensitivity to hapten–protein complexes in CFA and tuberculin hypersensitivity are amenable to study. George and Vaughan [63] packed peritoneal exudate cells within a capillary tube and exposed the superficial layer of cells to antigen in a small chamber. The antigen did indeed restrain an outgrowth of the monocytes. The technique was picked up by David and co-workers [64] and later by Bloom. David et al used the hapten–carrier system and easily confirmed the findings of Gell and Benacerraf about carrier specificity [64]; Bloom and Bennett [65] used the tuberculin system. Working separately, each soon found that the inhibition of migration was due to a soluble agent, which is not preformed within the cells [65–68]. Only the lymphocytes in the system had to be derived from sensitized animals; macrophages from a normal animal would serve. Thus an examination of culture supernatants containing lymphocyte metabolites was started. The soluble material was called macrophage inhibition factor (MIF). With application of different techniques by various workers, a large variety of "factors" has now been found, collectively termed "lymphokines" by Dudley Dumonde. Crude "MIF" is all of these. Actual MIF is only one of these. There are separate chemotactic factors for PMNs, monocytes, and, if antigen is present, for eosinophils. Factors that inhibit the proliferation of the cloning of HeLa cells are reported. There is also an inhibitor of DNA synthesis, a toxin for mouse L-cells, and a blastogenic factor. About 5 of a long list are identifiably separate factors. The mediators bind to the target cells; the binding site for MIF on macrophages possesses a terminal glucose residue. If culture filtrates are highly concentrated and injected intradermally into a naive guinea pig, a faintly pink swelling appears in about 4 hr and soon recedes. It has been bravely termed "instant hypersensitivity."

Since guinea-pig monocytes respond to culture filtrates of human cells incubated with antigen, an "indirect" MIF test is often used to study human lymphocytes. Still other techniques for performing the MIF test have been developed, including those which, in place of capillary tubes, use an assay of spreading on agar or on carboxymethyl cellulose "gum."

Another powerful tool for studying D-H in vitro is to expose cells to antigen in tissue culture for about 5 days. Blast forms, growing rapidly, can be seen microscopically or assessed by incorporation of a terminal pulse of [^{3}H]thymidine [69]. Adequate controls are needed to properly assess the blastogenic effects.

In vitro tests with cells taken from contactant type D-H have posed a problem because we do not have a proper carrier for haptens. Tests for MIF production have failed even though a wide variety of DNP-products, including DNP-guinea pig microsomes, has been tried. But recently blast transformation has been achieved by Levis [70,71], who coupled DNCB to human erythrocytes by means of dimethylsulfoxide (DMSO). In DNCB-sensitized rabbits, erythrocyte carriers similarly coupled, whether in rabbit, rat, or guinea pig, have stimulated cells to blastogenesis.

Lymphocyte Types

Since T-lymphocytes and B-lymphocytes (thymus-derived, bone marrow-derived) have been recognized as separate types, numerous studies have shown interaction between the two. The B-lymphocyte, which is capable of synthesizing specific antibody, can restrain T-cells, while T-cells can function as "helper" cells for B-cells in this synthesis; T-cells can also restrain B-cells. Obviously there are several subpopulations of T-cells. One or more of these subpopulations represent the effector of D-H. For certain studies, mice with visible "theta" markers on their T-cells, as well as the "nude mouse," which is genetically athymic, have proved useful.

The lymphocyte types—B, T, and the newly discovered Null cells—must be separated for definitive studies. The properties of these three types are given in Table II. None of them can phagocytize; B-cells carry surface immunoglobulins; T-cells rosette directly with sheep erythrocytes, but B-cells do so only when antibody and complement are present (erythrocyte–antibody–complement (EAC) rosettes). All three types respond to mitogens of some class. The newly discovered fact that B-cells produce much more MIF than T-cells has discouraged many workers who no longer believe that MIF production is a useful correlate of D-H. But in my experience [22], tests with the peritoneal exudate cells of guinea pigs are reliable since B-cells are not likely to be present. Alternatively, B-cells can be removed on an immunoadsorbent column bearing rabbit anti-IgG. Cellular proliferation is not necessary for MIF production by B-cells which continues when the cells are restrained by bromouridine (BUdR) with light. Properties that

TABLE II. *Properties of lymphocyte types*[a]

Thymus-derived (T) cells, bone-marrow-derived (B) cells, and the newly recognized class of "Null" lymphocytes are differentiable as summarized in the text. Abbreviations: Surface Ig, immunoglobulin bound to and forming part of the cell surface; E, (sheep) erythrocytes; EAC, (sheep) erythrocytes plus antisheep antibody plus complement; PHA, phytohemagglutinin; Con-A, concavalin A; Hu Ig, human immunoglobulin; MIF, macrophage inhibiting factor; Ag, antigen; MLC, mixed lymphocyte reaction (see text).

Antibody-dependent cell-mediated cytotoxicity refers to the ability of lymphocytes to lyse fresh chicken red cells which have been coated with Hu Ig or rabbit Ig or rabbit anti-Hu Ig at a killer-to-target cell ratio of 15:1.

	T	Null	B
Latex ingestion	0	0 (2%)	0
Surface Ig	0	0	+
E-rosettes	+	0	0
EAC-rosettes	0	+	+
Nylon adherence	0	0	+
PHA/Con-A/pokeweed	+++	+	+
Blasts via mitogen	No Ig		Surface Ig
Hu Ig-chick RBC[b]	0	+	++
MIF via Ag	+		+++ (BUDR + light also)
Interferon	+		+
Chemotactic factor	+		+
[^{3}H]Thymidine + Ag	+	0	0
Mitogenic factor	+ (on B-cells)		0
One-way MLC	+ (killers generated)	0	0

[a] Data rearranged from Chess et al [72]

[b] "Antibody-dependent cell-mediated cytotoxicity (ADCC)"

are unique to T-cells are direct rosetting with sheep erythrocytes, thymidine incorporation by sensitized cells in the presence of antigen, and the ability to become aggressive in one-way mixed lymphocyte cultures (MLC). (The target cells are restrained by actinomycin or mitomycin C: the lymphocytes under test develop killer cells, which can later be harvested and used to speedily attack other cells of the target provenance.) T-cells cannot lyse chicken erythrocytes coated with specific immunoglobulin in contrast to B-cells and Null cells. The mitogen factor which is active on B-cells appears only in the media of cultured T-cells.

Separation of the three types of lymphocytes depends upon the properties shown in the upper section of Table II. B-cells are first removed on a solid immunoadsorbent bearing Fab fragments prepared from antihuman immunoglobulin (Ig). Later they are harvested by displacement from the column with human Ig. The other cells are recovered from the wash-through, which is divided into two portions. One is processed for T-cells by EAC rosetting of Null cells and any remaining B-cells, the rosettes are removed on a density gradient of Hypaque–Ficoll, and the contaminating monocytes are trapped by adherence to cotton wool. The other portion is processed in reverse to obtain the Null cells: E-rosettes are removed on the density gradient and the monocytes on cotton wool [72].

Gell and Godfrey [73] extracted from the total T-cells those which are specifically committed to function in DNP-contact tests by first binding the cells to DNP-bearing polyacrylamide columns and then discharging them upon passage of DNP-amino acids. These committed cells appear to differ from the E-rosetting cells.

Macrophage Response to Lymphokines

Confined within chambers and exposed to increasing concentrations of lymphocyte metabolites, macrophages are paralyzed. But in vivo, antigen introduced into a sensitized animal produces dramatic changes: the macrophages enlarge greatly, metabolize with new energy, spread readily on glass, and acquire a marked propensity for phagocytosis. Such cells (Figs. 2, 3), which are termed "angry macrophages," have been studied by Blanden, Lefford, and Mackaness [74] and by others. Any one of several specific D-H systems leads to excitation, but antigen must be provided periodically to sustain the effect. Since the "angry macrophage" has a catholic taste for foreign invading bacteria of any sort, the animal can escape death from microbes entirely unrelated to its immunologic sensitization [60–62].

THE UNRESPONSIVE STATE (TOLERANCE)

By 1946, it was known that feeding a sensitizing chemical to a naive animal would abort or markedly reduce the development of D-H if an attempt was later made to sensitize with the same com-

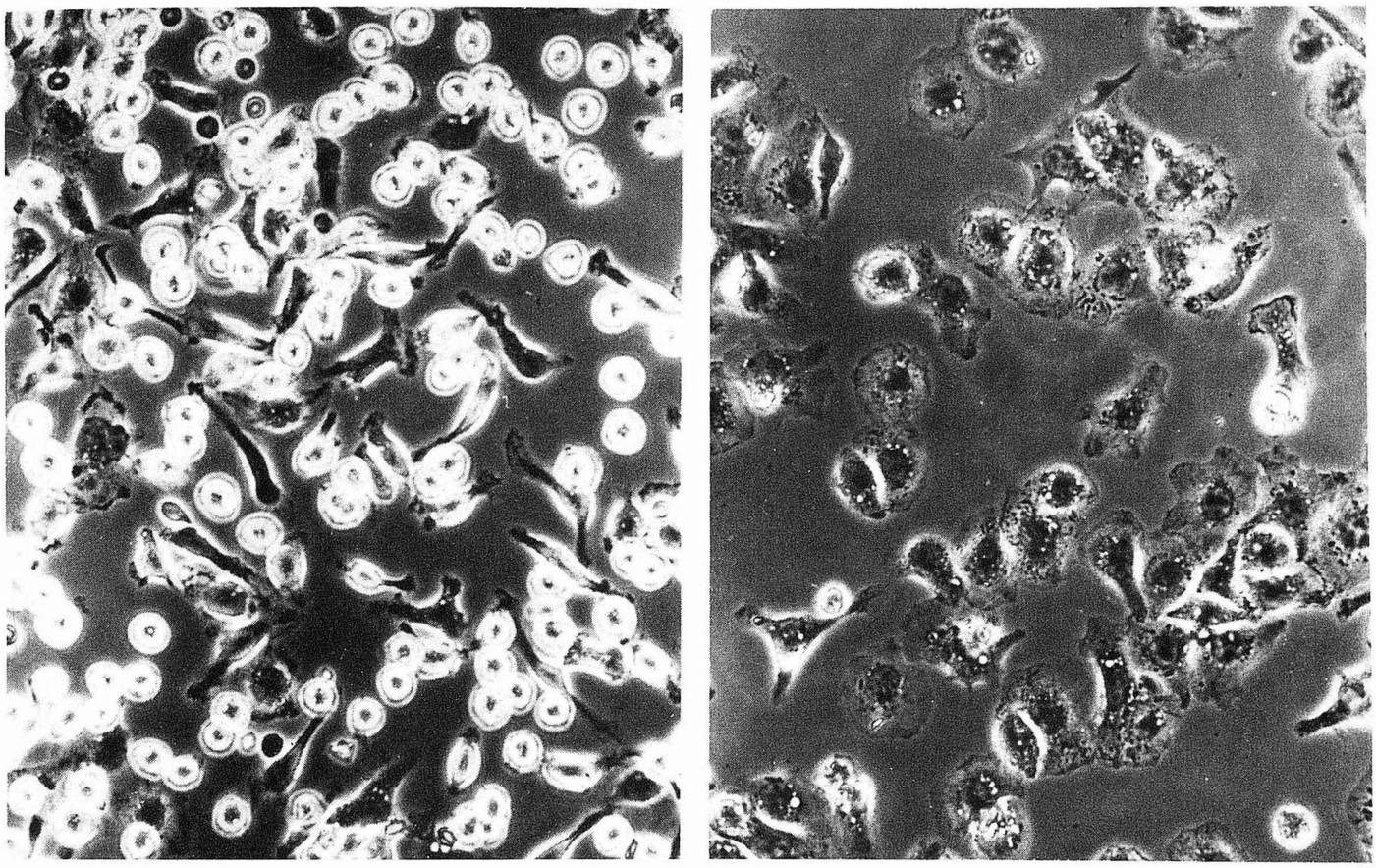

FIG. 2. The effect on macrophages of sensitization and restimulation. Peritoneal cells from mice sensitized with 1×10^4 living BCG cells 25 days before. The cells from such a mouse (*left panel*) were photographed after 3 hr of incubation. Few of the cells have spread; most remain rounded up. Cells in the *right panel* are from a mouse reinjected 48 hr before with 1×10^7 living BCG; the picture was taken after only 15 min to show the rapid spread on glass and the prominent mitochondria.

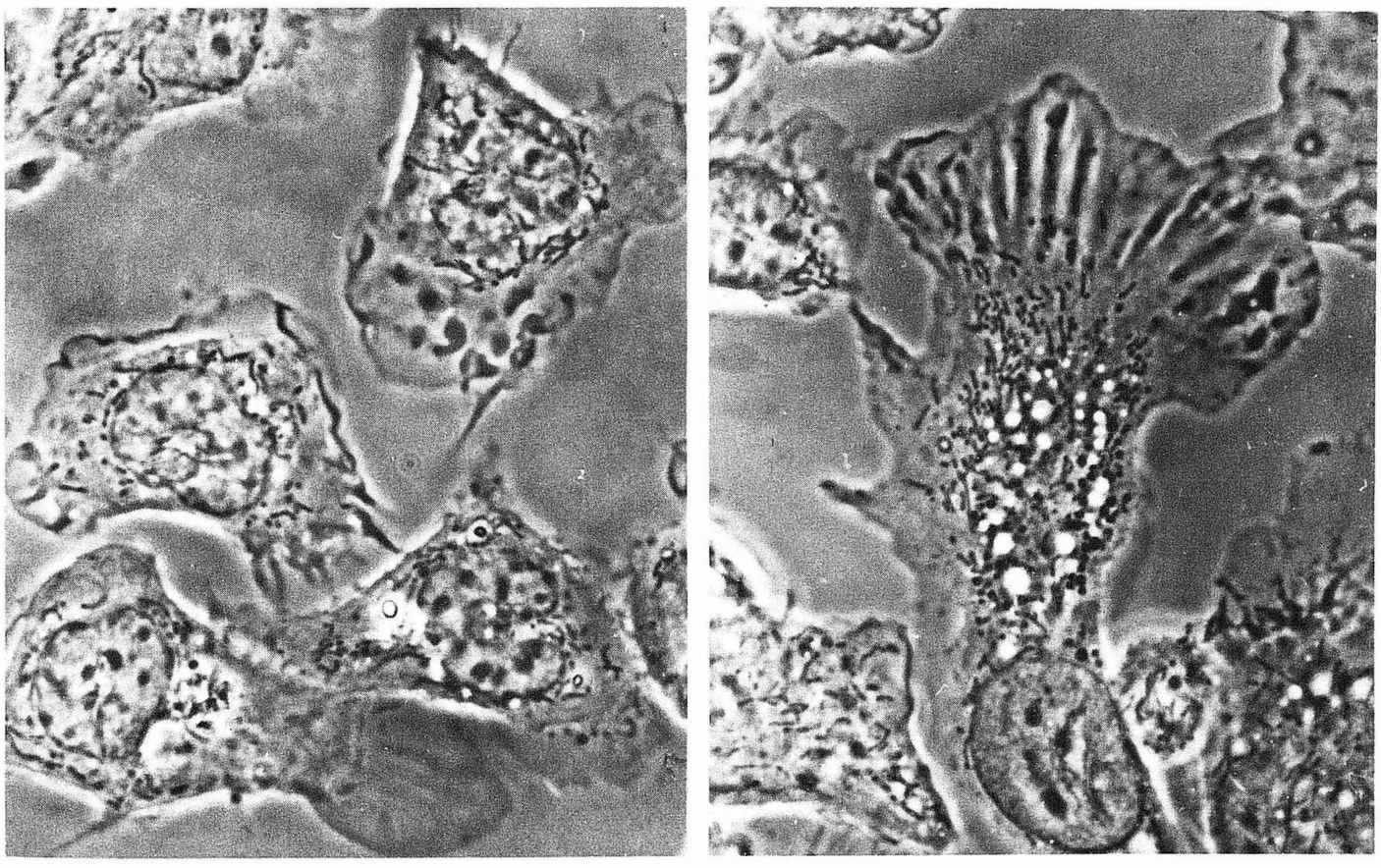

FIG. 3. *Left panel*: Peritoneal cells from a normal mouse in tissue culture for 24 hr, a time sufficient for spreading. *Right panel*: Cells from a BCG-infected mouse after reinjection, cultured similarly; about two-thirds of one macrophage occupies the center. Compared with the left-hand panel, there is an increased tendency to spread on glass; mitochondria are filamentous; phase-dense lysosomes are numerous; the phase-lucent vesicles indicate active pinocytosis. (After Blanden, Lefford, and Mackaness [74])

pound [75]. The unresponsiveness was shown to be specific for the compound fed; moreover, the synthesis of IgG_1 antibody, which the active sensitizing treatment was expected to initiate, was markedly depressed, i.e., anaphylaxis did not occur after the intravenous injection of hapten–protein complex. If more stringent sensitizing procedures were used, hapten-specific antibody did become synthesized, but the sensitivity to contact testing was elevated only slightly [76,77]. The system, highly effective in guinea pigs with haptens which are soluble in triglyceride oil, failed in man even when aqueous dilutions of tincture of Krameria were fed repeatedly and a sequence of patch tests to induce sensitivity was applied [78]. The subject was not pursued in man for some years.

Pomeranz and Norman [79,80] resumed the study with picryl chloride in guinea pigs. They used a single large intragastric feeding and documented the time of onset of tolerance. Lowney then induced tolerance in guinea pigs with *p*-nitrosodimethylaniline (NDMA) and later cautiously experimented with 97 human volunteers with DNCB [81]. After 7 patterns of treatment, Lowney concluded that unresponsiveness arises from repeatedly applying either 50 μg DNCB over 20 to 40 contact sites at one time (6 out of 16 subjects became solidly tolerant) or larger doses of DNCB on the buccal mucosa (Fig. 4). The latter method shows promise.

In animal models, however, a greater unresponsiveness to DNCB was developed by Frey, deWeck, and Geleick [82,83] who used a very large intravenous dose of dinitrobenzenesulfonate, a water-soluble substance of low toxicity. This method also served to desensitize a sensitized animal provided an intradermal injection of DNCB was given within 12 to 24 hr afterwards.

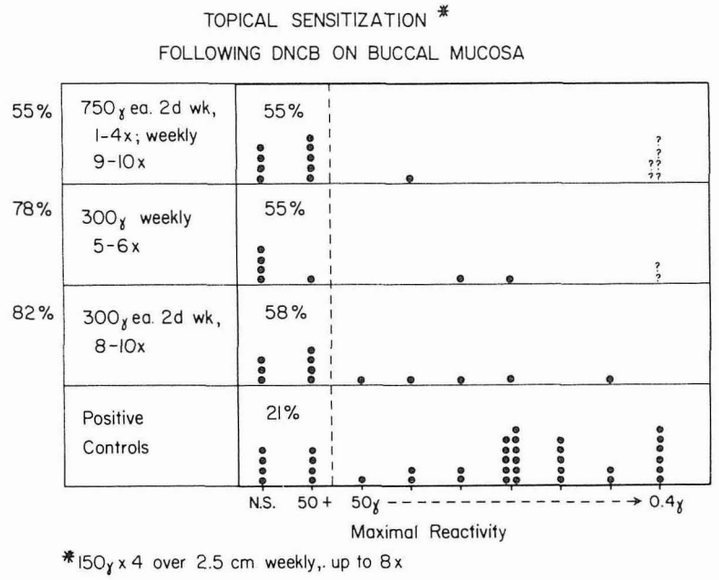

FIG. 4. Each *closed circle* represents a volunteer. After several procedures, all subjects were given the sensitizing course listed. Final titrations of reactivity are shown; the percentage of persons remaining in the program (some withdrew) are shown in the *left margin*. Persons rated as negative or trivial reactors are located on the *left side of the dashed line*, being either nonsensitive or minimally reactive to test concentrations greater than 50 μg. The sensitizing procedure failed to sensitize 21% of the control group, but this percentage was raised to 55–58% in the experimental groups. (Rearranged from data of E. M. Lowney, J Allergy Clin Immunol 48: 28, 1971)

Whatever the procedure, tolerance can be built up in adult animals only if the modes of application avoid early stimulation of the antibody-forming apparatus. When cyclophosphamide, which is known to restrain B-cells, was used to cover guinea pigs during the administration of antigenic hapten–protein complexes, Salvin and Smith [84] established tolerance. Tolerance involves a restraint of some populations of T-cells which extends to normal cells newly introduced into a tolerant animal. Parabiosis of a normal and tolerant guinea pig for 10 days rendered it completely unresponsive [85].

Animals thus rendered tolerant are widely used in special studies. In the experiments of Polak and Macher [53], lymphocytes exposed in vitro to small concentrations of DNCB had to be injected living into recipients even though the complete removal of the free chemical could not be assured. Accordingly, the recipients were made deeply tolerant to this allergenic chemical by a previous intravenous injection of DNP-sulfonate; therefore, the effects could not be ascribed to an active sensitization.

TRANSFER FACTOR

Transfer factor (TF) is an immunologic mystery, but so was IgE to immunologists once. In some fashion, this low-molecular-weight material exerts control over lymphocytes. TF was discovered around 1954 and has been studied largely by H. S. Lawrence and his colleagues; their work over the years is recounted in [86]. In the earlier years, a crude material was secured from peripheral lymphocytes of sensitized human beings either by (a) freezing and thawing, followed by digestion of nuclear material by DNase-plus-$MgSO_4$, (b) incubating cells in a relevant antigen such as tuberculin, or (c) shaking with glass beads. The dialyzable nature of TF was discovered in the early 1960s, and TF_D is now universally used, since the transplantation antigens, which can sensitize the recipient, remain in the retentate. Dialysates are now usually lyophilized.

TF_D can be processed on Sephadex G25 and G10 and on polyacrylamide to remove an inhibitor of unknown type and to permit search for the active fraction. The molecular weight of the dialyzing material is estimated as less than 4000 daltons. One "unit" is based on 5×10^8 lymphocytes in the starting material, or, in England, on 1×10^9 lymphocytes. There is as yet no unit of potency, which varies directly with the hypersensitivity of the donor. Because of the salts present, the weight of the material recovered in the dialysate is not known.

Since adult human beings have developed various types of D-H to infectious agents [88], all preparations of TF_D possess an assortment of TFs. Selective TF_Ds are made from donors highly sensi-

tive to the property that is to be transferred. (An extensive bibliography is given in [86] and recent studies in [89–91].)

Injection of TF_D from a donor highly sensitive to tuberculin will convert a pretested, normal recipient to tuberculin sensitivity, a state which can be detected shortly and apparently matures within 4 or 5 days and which leaves the recipient sensitive for many months or years. Pretesting and posttesting the recipient with a battery of recall antigens discloses other types of D-H which may be contained in the preparation used. TF_D, then, provides some sort of immunologic fillip. But TF exists in man only for microbial, fungal, and viral agents; it has never been reported on the lymphocytes of patients with allergic contact dermatitis. This is not surprising because cellular transfer of contact hypersensitivity is much more difficult to achieve in man than in the guinea pig. Despite its very small size and some conflicting data, TF_D generally conveys specific information to the recipient. A most important recent finding by Ascher et al [92] suggests new ways to evaluate TF_D in vitro. Rather than conducting dialysis into water and lyophilizing, the lysate is now dialyzed directly into a small volume of Medium 199, which is used directly. This slight technical alteration yields a TF_{DM} which can instruct naive human lymphocytes to react to specific antigen and to incorporate thymidine. The blastogenesis may involve a 2-stage effect, such as an initial triggering by TF_{DM} that provokes cellular synthesis of a nondialyzable factor which then leads to blastogenesis. Cultured lymphoblastoid cells from a nonsensitive human donor are reported to respond to TF_D by synthesizing TF through the B-cells in the preparation [90].

In recent years, the "re-arming" of patients with D-H immunodeficiencies by means of TF_D has been reported to be beneficial. Since such patients do not retain the newly acquired hypersensitivity the same way a normal recipient does, repeated injections are necessary. In pilot studies, TF_D from highly chosen donors or from donors possessing other specificities of TF_Ds has been given to about 225 patients in many disease categories such as Wiskott–Aldrich syndrome, chronic candidiasis, acute malignant measles, congenital rubella, acute rheumatoid arthritis, osteogenic sarcoma, acute leukemia, leprosy, coccidioidomycosis, multiple sclerosis, and subacute sclerosing panencephalitis. Many patients acquire, at least temporarily, skin reactivity to test antigens and in vitro antigen-induced blastogenesis. Up to 30% or so patients may show some degree of clinical improvement, sometimes for a few months. Such improvement, when it comes, is said to be evident after the initial injections. Double-blind studies are needed to evaluate the actual therapeutic effect of TF_D and to determine whether specificity (TF_{D+}) or only "broad spectrum" effects ($TF_{D(-)}$) are necessary. Such studies are being planned for leprosy and for coccidioidomycosis.

If the benefits which derive from the use of $TF_{D(-)}$) are tested by sequential skin tests with a battery of recall antigens, they could be attributed to the provoking of "angry macrophages" by re-armed, antigen-stimulated lymphocytes, which thereafter become nonselective in the foreign elements which they attack.

THE FUTURE?

Cell biologists and immunologists will surely lead in classifying the subpopulations of B- and T-cells for which we have only indirect evidence so far. Much new data can be derived from a study of the interactions between the various cell types, including basophils and eosinophils. But within the sensitized individual, each testing will initiate the same complex, intertwined events. Surely the scanning electron microscope, which utilizes thicker sections, can be expected to show how intimately the invading cells are packed against one another and against the basal epidermal cells in which spongiosis occurs.

We know that ultimately the package of lymphokines will be broken open to reveal the actual number and relative importance of the components and that probably the metabolites of macrophages and basophils can be examined as well.

The biologic problem of TF, which has been subverted by the lurch to therapeutic use in patients, deserves a much better experimental design. Is it really narrowly specific, and if so, how is this property possible in so small a molecule? Can it be secured in weighable amounts, free from magnesium sulfate in the dialysate? Can its activity be standardized? Does it arise from macrophages or B-cells and indirectly activate the T-cells from which it is harvested? Is it adsorbed by purified T-cells? Is there a TF_D which converts normal human subjects into contact-sensitive patients? Will TF_D function if recipients are injected with test antigen 6 months before the injection of TF_D and are not tested until 3 more months have elapsed? In other words, does antigen play a role when given shortly before or after TF?

So far no animal model has been found in which to study TF under controlled experimental conditions. Even if animals have now and then shown some evidence of TF_D, we still need a system that yields reproducible results and material of good activity before we can replace man as the primary test subject. Further work may reveal this sorely needed model.

Studies in physiology are needed to provide us with a better understanding of the events begun by immunologic triggering. Why, for example, does a sensitized guinea pig respond so poorly on the day of or on the day after the drawing of moderate amounts of blood? The proportion of T-cells withdrawn by the bleeding must be trivial compared with the reserves left in the blood, nodes, and spleen. We cannot escape the conclusion that we still have much to learn and that we will do so only through new modes of experimentation.

REFERENCES

1. Landsteiner K, Jacobs JL: Studies on the sensitization of animals with simple chemical compounds. II. J Exp Med 64:625–639, 1936
2. Eisen HN, Orris L, Belman S: Elicitation of delayed allergic skin reaction with haptens: the dependence of elicitation on hapten combination with proteins. J Exp Med 95:473–487, 1952
3. Landsteiner K, Chase MW: Experiments on transfer of cutaneous sensitivity to simple chemical compounds. Proc Soc Exp Biol Med 49:688–690, 1942
4. Chase MW: Cellular transfer of cutaneous hypersensitivity to tuberculin. Proc Soc Exp Biol Med 59:134–135, 1945
5. Urbach E: Zur Pathogenese der cutanen Idiosynkrasien. Arch f Dermat u Syph 148:146–157, 1924
6. Freund J: Some aspects of active immunication. Annu Rev Microbiol 1:291–308, 1947
7. Coulaud E: Etat allergique durable, obtenu chez les animaux de laboratoire, par injection sous-cutanée de bacilles tuberculeux morts, enrobés dans la paraffine solide. Rev Tuberc (Paris) 2:850–855, 1934
8. Saenz A: Etat d'allergie intense, rapide et durable, confére au cobaye par ingestion de bacilles tuberculeux morts enrobés dans de l'huile de vaseline; son mecanisme. C R Soc Biol (Paris) 120:870–873, 1935
9. Saenz A: Accroissement de l'état allergique et titrage de la sensibilité tuberculinique conferés au cobaye par l'inoculation sous-cutanée de bacilles tuberculeux morts enrobés dans de l'huile de vaseline. C R Soc Biol (Paris) 120:1050–1053, 1935
10. Dienes L, Schoenheit EW: Certain characteristics of the infectious processes in connection with the influence exerted on the immunity response. J Immunol 19:41–61, 1930
11. Freund J, McDermott K: Sensitization to horse serum by means of adjuvants. Proc Soc Exp Biol Med 49:548–553, 1942
12. Chase MW: Experimental sensitization with particular reference to picryl chloride. Int Arch Allergy Appl Immunol 5:163–191, 1954
13. Turk JL: Delayed hypersensitivity, Frontiers of Biology, vol 4. Edited by EA Neuberger, EL Tatum. Amsterdam, North-Holland, 1967, pp 80–87, 98–99
14. Friedlaender MH, Chisari FV, Baer H: The role of the inflammatory response of skin and lymph nodes in the induction of sensitization to simple chemicals. J Immunol 111:164–170, 1973
15. Magnusson B, Kligman AM: Allergic contact dermatitis in the guinea pig, Identifications of Contact Allergens. Springfield, Ill, Thomas, 1970, 141 pp
16. Maguire HC Jr: The bioassay of contact allergens in the guinea pig. J Soc Cosmet Chem 24:151–162, 1973
17. Chase MW, Macher E: The fate of antigen in induction of delayed allergic responses, Advances in Biology of Skin, vol XI, Immunology and the Skin. Edited by W Montagna, RE Billingham. New York, Appleton-Century-Crofts, 1971, pp 63–93
18. Maguire HC Jr: Alteration in the acquisition of delayed hypersensitivity with adjuvant in the guinea pig, Monographs in Allergy, vol 8. Edited by D Parker, JL Turk. New York, Karger, 1974, pp 13–26
19. Brent L, Brown JB, Medawar PE: Skin transplantation immunity in relation to hypersensitivity reactions of the delayed type. Lancet 2:561–564, 1958
20. Brent L, Brown JB, Medawar PE: Quantitative studies on tissue transplantation immunity. VI. Hypersensitivity reactions associated with the rejection of homografts. Proc R Soc Lond [Biol] 56:187–209, 1962
21. Billingham RE: Transplantation immunity evoked by skin homografts and expressed in intact skin, Advances in Biology of Skin, vol XI, Immunology and the Skin. Edited by W Montagna, RE Billingham. New York, Appleton-Century-Crofts, 1971, pp 183–198
22. Chase MW: Hypersensitivity to simple chemicals, Harvey Lectures Series 61. New York, Academic, 1967, pp 169–203
23. Maguire HC Jr, Chase MW: Exaggerated delayed-type hypersensitivity to simple chemical allergens in the guinea pig. J Invest Dermatol 49:460–468, 1967
24. Maguire HC Jr, Chase MW: Studies on the sensitization of animals with simple chemical compounds. XIII. Sensitization of guinea pigs with picric acid. J Exp Med 135:357–375, 1972
25. Chase MW, Maguire HC Jr: Studies on the sensitization of animals with simple chemical compounds. XIV. Further studies on sensitization of guinea pigs with picric acid. Int Arch Allergy Appl Immunol 45:513–542, 1973
26. Macher E, Dörner V: Delayed-type contactant hypersensitivity elicited in epithelia of vagina, uterus and colon of the guinea pig. J Immunol 97:484–491, 1966
27. Fisher JP, Cooke RA: Experimental toxic and allergic contact dermatitis. J Allergy 29:411–428, 1958
28. Richerson HB, Dvorak HF, Leskowitz S: Cutaneous basophil hypersensitivity. I. A new look at the Jones-Mote reaction; general characteristics. J Exp Med 132:546–557, 1970
29. Dvorak HF, Simpson BA, Bast RC Jr, Leskowitz S: Cutaneous basophil hypersensitivity. III. Participation of the basophil in hypersensitivity to antigen–antibody complexes, delayed hypersensitivity and contact allergy. Passive transfer. J Immunol 107:138–148, 1971
30. Wolf-Jürgensen P: Basophilic Leukocytes in Delayed Hypersensitivity. Experimental Studies in Man Using the Skin Wound Technique. Copenhagen, Munksgaard, 1966, 123 pp
31. Colvin RB, Johnson RA, Mihm MC Jr, Dvorak HF: Role of the clotting system in cell-mediated hypersensitivity. I. Fibrin deposition in delayed skin reactions in man. J Exp Med 138:686–698, 1973
32. Dvorak HF, Mihm MC Jr, Dvorak AM, Johnson RA, Manseau EJ, Morgan E, Colvin RB: Morphology of delayed type hypersensitivity reactions in man. I. Quantatitive description of the inflammatory response. Lab Invest 31:111–130, 1974
33. Dvorak HF, Selvaggio SS, Dvorak AM, Colvin RB, Lean DB, Rypyse J: Purification of basophilic leukocytes from guinea pig blood and bone marrow. J Immunol 113:1694–1702, 1974
34. Chase MW: Delayed sensitivity, Basic and Clinical Immunology. Med Clin North Am 49:1613–1646, 1965
35. Katz SI, Heather CJ, Parker D, Turk JL: Basophilic leukocytes in delayed hypersensitivity reactions. J Immunol 113:1073–1078, 1974
36. Turk JL: Delayed hypersensitivity, Frontiers of Biology, vol 4. Edited by EA Neuberger, EL Tatum. Amsterdam, North-Holland, 1967, 252 pp
37. Turk JL, Parker D: B-cell modulation of T-cell function in delayed hypersensitivity with special reference to contact sensitivity, Monographs in Allergy, vol 8. Edited by D Parker, JL Turk. New York, Karger, 1974, pp 44–53
38. Ishizaka K, Ishizaka T: IgE and reaginic hypersensitivity. Ann NY Acad Sci 190:443–456, 1971
39. Askenase P: Cutaneous basophil hypersensitivity in contact-sensitized guinea pigs. J Exp Med 138:1144–1155, 1973
40. Salvin SB, Smith RF: The specificity of allergic reactions. III. Contact hypersensitivity. J Exp Med 114:185–194, 1961
41. Salvin SB: Roles of haptens and carriers in delayed allergies, Advances in Biology of Skin, vol XI, Immunology and the Skin. Edited by W. Mon-

tagna, RE Billingham. New York, Appleton-Century-Crofts, 1971, pp 95–101
42. Chase MW, Kawata H: Induction of contact sensitivity by hapten-epithelium conjugates. J Invest Dermatol 56:255, 1971 and Clin Res 19:578, 1971
43. Landsteiner K, Chase MW: Studies on the sensitization of animals with simple chemical compounds. VI. Experiments on the sensitization of guinea pigs to poison ivy. J Exp Med 69:767–784, 1939
44. Frey JR, Wenk P: Experimental studies on the pathogenesis of contact eczema in the guinea pig. Int Arch Allergy Appl Immunol 11:81–100, 1957
45. Turk JL, Stone SH: Implications of the cellular changes in lymph nodes during the development and inhibition of delayed-type hypersensitivity, Cell-Bound Antibodies. Edited by B Amos, H Koprowski. Philadelphia, Wistar Institute Press, 1963, pp 51–60
46. Turk JL: The cytologic bases of immune responses, Advances in Biology of Skin, vol XI, Immunology and the Skin. Edited by W Montagna, RE Billingham. New York, Appleton-Century-Crofts, 1971, pp 45–61
47. Macher E, Chase MW: Studies on the sensitization of animals with simple chemical compounds. XI. The fate of labeled picryl chloride and dinitrochlorobenzene after sensitizing injections. J Exp Med 129:81–102, 1969
48. Macher E, Chase MW: Studies on the sensitization of animals with simple chemical compounds. XII. The influence of excision of allergenic depots on onset of delayed sensitivity and tolerance. J Exp Med 129:103–121, 1969
49. Godfrey HP, Baer H, Watkins RC: Delayed hypersensitivity to catechols. V. Absorption and distribution of substances related to poison ivy extracts and their relation to the induction of sensitization and tolerance. J Immunol 106:91–102, 1971
50. Medawar PB: The homograft reaction. Proc R Soc Lond [Biol] 149:145–166, 1958
51. Barker CF: Lymphatics and homograft reaction and the role of peripheral sensitization, Advances in Biology of Skin, vol XI, Immunology and the Skin. Edited by W Montagna, RE Billingham. New York, Appleton-Century-Crofts, 1971, pp 199–211
52. Macher E, Sommer G: Attemps to induce contact hypersensitivity *in vitro*, Contact Hypersensitivity in Experimental Animals, Monographs in Allergy, vol 8. Edited by D Parker, JL Turk. New York, Karger, 1974, pp 110–119
53. Polak L, Macher E: *In vitro* sensitization to dinitrochlorobenzene in guinea pigs. Nature (Lond) 252:748–749, 1974
54. Frey JR, deWeck AL, Geleich H, Lergier W: The immunogenicity of dinitrophenylated amino acids. J Exp Med 130:1123–1143, 1969
55. Benacerraf B, Gell PGH: Studies on hypersensitivity. I. Delayed and Arthus-type skin reactivity to protein conjugates in guinea pigs. Immunology 2:53–63, 1959
56. David JR, Al-Askari S, Lawrence HS, Thomas L: Delayed hypersensitivity *in vitro*. I. The specificity of inhibition of cell migration by antigens. J Immunol 93:264–273, 1964
57. Kirchheimer WF, Weiser RS: The tuberculin reaction. I. Passive transfer of tuberculin sensitivity with cells of tuberculous guinea pigs. Proc Soc Exp Biol Med 66:166–170, 1947
58. Wesslén T: Passive transfer of tuberculin hypersensitivity by viable lymphocytes from the thoracic duct. Acta Tuberc Scand 26:36–53, 1952
59. Hensley GT, Fink JN, Barboriak JJ: Hypersensitivity pneumonitis in the monkey. Arch Pathol 97:33–38, 1974
60. Mackaness GB: Cellular resistance to infection. J Exp Med 116:381–406, 1962
61. Mackaness GB: Immunological basis of acquired cellular resistance. J Exp Med 120:105–120, 1964
62. World Health Organization, Technical Report Series, No 519. Cell-mediated immunity and resistance to infection: report of a WHO scientific group, Geneva, 1973
63. George M, Vaughan JH: *In vitro* cell migration as a model for delayed hypersensitivity. Proc Soc Exp Biol Med 111:514–521, 1962
64. David JR, Al-Askari S, Lawrence HS, Thomas L: Delayed hypersensitivity *in vitro*. I. Specificity of inhibition of cell migration by antigen. J Immunol 93:264–273, 1964
65. Bloom BR, Bennett B: Mechanism of a reaction *in vitro* associated with delayed-type hypersensitivity. Science 153:80–82, 1966
66. Bennett B, Bloom BR: Studies on the migration inhibitory factor associated with delayed-type hypersensitivity. Cytodynamics and specificity. Transplantation 5:996–1000, 1967
67. David JR: Delayed hypersensitivity *in vitro:* its mediation by cell-free substances formed by lymphoid cell-antigen interaction. Proc Natl Acad Sci USA 56:72–77, 1966
68. Bennett B, Bloom BR: Reactions *in vivo* and *in vitro* produced by a soluble substance associated with delayed-type hypersensitivity. Proc Natl Acad Sci USA 59:756–762, 1968
69. Valentine FT: The transformation and proliferation of lymphocytes *in vitro*, Cell-Mediated Immunity, *In Vitro* Correlates. Edited by JP Revillard. New York, Karger/University Park Press, 1971, pp 6–42
70. Chase MW, Hunziger N. Rapporteurs of Workshop No. 73: Contact sensitisation, Progress in Immunology II, vol 3. Edited by L. Brent, J Holborow. Amsterdam, North-Holland, 1974, pp 369–372
71. Levis WR, Powell JA, Whalen JJ: Carrier specificity vs hapten specificity in classical cell-mediated contact sensitivity to dinitrochlorobenzene. J Immunol 115:1170–1173, 1975
72. Chess L, MacDermott RP, Sondel PN, Schlossman SF: Isolation and characterization of cells involved in human cellular hypersensitivity, Progress in Immunology II, vol 3. Edited by L Brent, J Holborow. New York, North-Holland/American Elsevier, 1974, pp 125–132
73. Gell PGH, Godfrey HP: Studies in cell separation in delayed hypersensitivity, Contact Hypersensitivity in Experimental Animals, Monographs in Allergy, vol 8. Edited by D Parker, JL Turk. New York, Karger, 1974, pp 89–99
74. Blanden RV, Lefford MJ, Mackaness G: The host response to Calmette-Guérin bacillus infection in mice. J Exp Med 129:1079–1107, 1969
75. Chase MW: Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. Proc Soc Exp Biol Med 61:257–259, 1946
76. Battisto JR, Chase MW: Induced unresponsiveness to simple allergenic chemicals. II. Independence of delayed-type hypersensitivity and formation of circulating antibody. J Exp Med 121:591–606, 1965
77. Chase MW, Battisto JR, Ritts RE: Acquisition of immunologic tolerance via simple allergenic chemicals, Conceptual Advances in Immunology and Oncology. New York, Hoeber-Harper, 1963, pp 395–416
78. Grolnick M: Studies in contact dermatitis. VIII. The effect of feeding antigen on the subsequent development of skin sensitization. J Allergy 22:170–174, 1951
79. Pomeranz JR, Norman PS: Concomitant anaphylactic sensitization and contact unresponsiveness following the infusion or feeding of picryl chloride to guinea pigs. J Exp Med 124:69–80, 1966
80. Pomeranz JR: Immunologic unresponsiveness following a single feeding of picryl chloride. J Immunol 104:1486–1490, 1970
81. Lowney EM: Tolerance of dinitrochlorobenzene, a contact sensitizer in man. J Allergy 48:28–35, 1971
82. Frey JR, deWeck AL, Geleick H: Inhibition of the

contact reaction to dinitrochlorobenzene by intravenous injection of dinitrobenzene sulfonate in guinea pigs sensitized to dinitrochlorobenzene. J Invest Dermatol 42:189–196, 1964
83. deWeck AL, Frey JR: Immunotolerance to Simple Chemicals, Monographs in Allergy, vol 1. New York, Karger/American Elsevier, 1966
84. Salvin SB, Smith RF: The specificity of allergic reactions. VII. Immunologic unresponsiveness, delayed hypersensitivity and circulating antibody to proteins and hapten-protein conjugates in adult guinea pigs. J Exp Med 119:851–868, 1964
85. Polak L: The transfer of tolerance to DNCB-contact sensitivity in guinea pigs by parabiosis. J Immunol 114:988–991, 1975
86. Lawrence HS: Transfer factor in cellular immunity. Harvey Lectures Series 68. New York, Academic, 1974, pp 239–350
87. Workshop on Basic Properties and Clinical Applications of Transfer Factor. Edited by CH Kirkpatrick, D Rifkind. Sponsored by the National Institute of Allergy and Infectious Diseases and the Department of Microbiology, University of Arizona College of Medicine, Tuscon, Arizona, February 16–17, 1973
88. Chase MW: Delayed-type hypersensitivity and the immunology of Hodgkin's disease, with a parallel examination of sarcoidosis. Cancer Res 26:1097–1120, 1966
89. Fudenberg HH: Transfer factor in immunodeficiencies, Progress in Immunology II, vol 5. Edited by L Brent, J Holborow. Amsterdam, North-Holland, 1974, 215–221
90. Gottlieb AA: Transfer factor: biological properties (Report of Workshop). Progress in Immunology II, vol 5. Amsterdam, North-Holland, 1974. pp 371–376
91. Valdimarsson H: Transfer factor, clinical application (Report of Workshop). Progress in Immunology, II, vol 5. Amsterdam, North-Holland, 1974, pp 377–382
92. Ascher MS, Schneider WJ, Valentine FT, Lawrence HS: *In vitro* properties of leukocyte dialysates containing transfer factor. Proc Natl Acad Sci USA 71:1178–1182, 1974